

$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_b \\ -\mathrm{GalNAc-(Gal)}_a - (\mathrm{Sia)}_c - (\mathrm{R})_d \end{bmatrix}_c$$

a-c, e (independently selected) = 0 or 1; R = modifying group, mannose, oligomannose.

FIG. 39A

CHO, BHK, 293 cells, Vero expressed IL-2 a-c, e (independently selected) = 0 or 1; d = 0

Sialidase
 CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

### FIG. 39B

Insect cell expressed IL-2 a, e (independently selected) = 0 or 1; b, c, d = 0.

Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1; R = PEG.

## 164/345

```
E. coli expressed IL-2
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0; R = PEG.
```

### FIG. 39D

```
NSO expressed IL-2
a, e (independently selected) = 0 or 1;
b, c, d = 0

1. CMP-SA-levulinate, ST3Gall
2. H_4N_2-PEG

a, c, d, e (independently selected) = 0 or 1;
b = 0; R = PEG.
```

## FIG. 39E

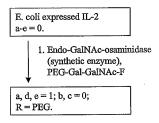


FIG. 39F

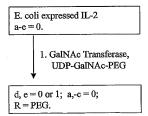


FIG. 39G

#### 166/345

2 peptides
A and A' - N-linked sites
B - O-linked sites

a-d, i, n-u (independently selected) = 0 or 1. aa, bb (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z = 0; R = polymer, glycoconjugate.

### **FIG. 40A**

#### 167/345

```
CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed Factor VIII. e-h = 1 to 4; aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1; v-z = 0.
```

```
    Sialidase
    CMP-SA-PEG, ST3Gal3
```

```
e-h = 1 to 4;
aa, bb, a-d, i, n, q-u (independently selected) = 0 or 1;
o, p, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

#### **FIG. 40B**

```
CHO, BHK, 293S cells, Vero, MDCK, 293S, HEKC expressed Factor VIII. e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;
v-z = 0.
```

```
1. Sialidase
2. CMP-SA-PEG, ST3Gal3
3. ST3Gal1, CMP-SA
```

```
e-h = 1 to 4;
aa, bb, a-d, i, n, p-u (independently selected) = 0 or 1;
o, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 40C

CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;
v-z = 0.

## 1. CMP-SA-PEG, ST3Gal3

e-h = 1 to 4; aa, bb, a-d, i, n-u (independently selected) = 0 or 1; z = 0; j-m, v-y (independently selected) = 0 or 1; R = PEG.

## FIG. 40D

CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected) 0 or 1;
v-z = 0.

## 1. CMP-SA-PEG, ST3Gal1

e-h = 1 to 4; aa, bb, a-d, i, n-u (independently selected) = 0 or 1; z = 0; j-m, v-y (independently selected) = 0 or 1; R = PEG.

#### FIG. 40E

#### 169/345

CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;
v-z = 0.

# 1. CMP-SA-PEG, α2,8-ST

e-h = 1 to 4; aa, bb, a-d, i, n-y (independently selected) = 0 or 1; z = 0; j-m (independently selected) = 0 to 2; v-y (independently selected) = 1, when j-m (independently selected) is 2; R = PEG.

## FIG. 40F

#### 170/345

2 peptides
A or A' - N-linked sites
B - O-linked sites

a-d, i, n-u, (independently selected) = 0 or 1.
aa, bb, cc, dd (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0;
R = modifying group, mannose, oligo-mannose.
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 40G

#### 171/345

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

1. CMP-SA-levulinate, ST3Gal3, 2. H<sub>4</sub>N<sub>2</sub>-PEG

```
e-h = 1 to 4;
aa, bb, cc, a-d, i, n-u (independently selected) = 0 or 1;
dd, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

## FIG. 40H

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

endo-H
 galactosyltransferase, UDP-Gal-PEG

```
e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z = 0; R' = -Gal-PEG.
```

## FIG. 401

#### 172/345

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC
expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.

1. ST3Gal3, CMP-SA
2. endo-H
3. galactosyltransferase, UDP-Gal-PEG
```

```
e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z=0; R'=-Gal-PEG.
```

## FIG. 40J

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

1. mannosidases
2. GNT 1 & 2, UDP-GlcNAc

3. galactosyltransferase, UDP-Gal-PEG

```
e-h = 1 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0; R = PEG.
```

#### FIG. 40K

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

```
1. mannosidases
```

- 2. GNT-1,2, 4 & 5; UDP-GlcNAc
- 3. galactosyltransferase, UDP-Gal
   4. ST3Gal3, CMP-SA

```
e-h = 1 to 4; aa, bb, cc, a-d, i, j-q (independently selected) = 0 or 1; dd, v-z=0.
```

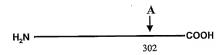
### FIG. 40L

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

```
    mannosidases
    GNT-1, UDP-GlcNAc-PEG
```

```
e-h = 0 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0.
```

#### FIG. 40M



$$\mathbf{A} = \underbrace{\left( \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{a} \end{bmatrix}_{a}^{-} \cdot \left( \operatorname{Sia} \right)_{j} - \left( \operatorname{R} \right)_{v} \right)_{r}^{-}}_{\left[ \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{b} \end{bmatrix}_{l}^{-} \cdot \left( \operatorname{Sia} \right)_{k} - \left( \operatorname{R} \right)_{w} \right]_{s}^{-}}_{\left[ \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \end{bmatrix}_{g}^{-} \cdot \left( \operatorname{Sia} \right)_{k} - \left( \operatorname{R} \right)_{x} \right)_{t}^{-}} \\ \underbrace{\left[ \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \end{bmatrix}_{g}^{-} \cdot \left( \operatorname{Sia} \right)_{u} - \left( \operatorname{R} \right)_{y} \right]_{u}^{-}}_{\left[ \underbrace{\left( \operatorname{GlcNAc-(Gal)}_{d} \right)_{g}^{-} \cdot \left( \operatorname{Sia} \right)_{m} - \left( \operatorname{R} \right)_{y} \right)_{u}^{-}}_{u} \right]_{t}^{-}}_{q}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 41A

#### 175/345

CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

## FIG. 41B

CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

```
1. Sialidase
2. CMP-SA-PEG (1.2 mol eq),
ST3Gal3
3. CMP-SA (16 mol eq), ST3Gal3
```

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

#### FIG. 41C

#### 176/345

```
NSO expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.
```

```
1. Sialidase and α-galactosidase
2. α-Galactosyltransferase, UDP-Gal
```

★ 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

## FIG. 41D

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.
```

```
1. Sialidase
2. CMP-SA-PEG (16 mol eq),
ST3Gal3
3. CMP-SA. ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

### FIG. 41E

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>A</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

### FIG. 41F

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

```
1. CMP-SA, α2,8-ST
```

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1;
j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

#### FIG. 41G



$$\mathbf{A} = (\operatorname{Fuc})_{i} \\ \operatorname{GlcNAc-\operatorname{Gal}}_{a}|_{e^{-}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v})_{i} \\ \operatorname{GlcNAc-\operatorname{Gal}}_{a}|_{e^{-}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v})_{i} \\ \operatorname{GlcNAc-\operatorname{Gal}}_{a}|_{g^{-}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v})_{i} \\ \operatorname{(R')}_{n} (\operatorname{GlcNAc-\operatorname{Gal}})_{d}|_{g^{-}} (\operatorname{Sia})_{i}^{-} (\operatorname{R})_{v})_{u} \\ \operatorname{(glcNAc-\operatorname{Gal}})_{d}|_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{v})_{u} \\ \operatorname{(glcNAc-\operatorname{Gal})_{d}} (\operatorname{Gal})_{u} (\operatorname$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 41H

### 179/345

```
Insect cell expressed Urokinase. a-d, f, h, j-n, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;
j-n = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

#### FIG. 411

```
Yeast expressed Urokinase.

a-n=0;

q-y (independently selected) = 0 to 1;

p=1; R (branched or linear) = Man, oligomannose.
```

```
    Endoglycanase
    Galactosyltransferase, UDP-Gal
    CMP-SA-PEG, ST3Gal3
```

```
a-m, p-y=0; n (independently selected) = 0 or 1;
R'=-Gal-Sia-PEG.
```

#### FIG. 41J

#### 180/345

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; n, v-y=0.
```

- CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated Urokinase produced in CHO.
- 3. CMP-SA, ST3Gal3, ST3Gal1

```
a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-Urokinase.
```

## FIG. 41K

```
Isolated Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0; n = 0; Sia (independently selected) = Sia or SO_4; Gal (independently selected) = Gal or GalNAc; GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.
```

sulfohydrolase
 CMP-SA-PEG, sialyltransferase

```
a-d, i-m, q-u (independently selected) = 0 or 1; 

n=0; e-h = 1; Sia = Sia; 

Gal (independently selected) = Gal or GalNAc; 

GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc. 

v-y (independently selected) = 0 or 1; 

R=PEG.
```

## FIG. 41L

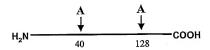
Isolated Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; n = 0; v-y = 0; Sia (independently selected) = Sia or  $SO_4$ ; Gal (independently selected) = Gal or GalNAc; GloNAc (independently selected) = GloNAc or GloNAc-Fuc.

1. sulfohydrolase, hexosaminidase

2. UDP-Gal-PEG, galactosyltransferase

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-n = 0; Gal (independently selected) = Gal; GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc; v-y (independently selected) = 0 or 1; R = PEG.

### FIG. 41M



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \text{(Fuc)}_{i} \\ \text{(GlcNAc-(Gal)}_{b}]_{c}^{-} \text{(Sia)}_{j} - (R)_{v} \\ \text{([GlcNAc-(Gal)}_{b}]_{r} - (Sia)_{k} - (R)_{w} \\ \text{([GlcNAc-(Gal)}_{d}]_{p}^{-} \text{(Sia)}_{l} - (R)_{x} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m} - (R)_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m} - (R)_{y} \\ \text{([GlcNAc-(Gal)}_{d}]_{h}^{-} \text{(Sia)}_{m} - (R)_{y} \\ \text{(Sia)}_{l} - (R)_{y} \\ \text{($$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 42A

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. Sialidase
2. CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

#### FIG. 42B

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (1.2 mol eq), ST3Gal3
    CMP-SA (16 mol eq), ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 42C

```
NSO expressed DNase I.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.
```

```
    Sialidase and α-galactosidase
    α-Galactosyltransferase, UDP-Gal
```

3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

#### FIG. 42D

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq), ST3Gal3
    CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 42E

CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N<sub>2</sub>-PEG
```

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

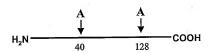
#### FIG. 42F

CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.



a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

## FIG. 42G



$$\mathbf{A} = \begin{bmatrix} (\operatorname{Fuc})_i \\ -\operatorname{GlcNAc-Man} \\ (R')_n \end{bmatrix} \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_a]_e^- & (\operatorname{Sia})_j^- & (R)_v \end{bmatrix}_r \\ (\operatorname{GlcNAc-(Gal)}_b]_f^- & (\operatorname{Sia})_k^- & (R)_w \end{bmatrix}_s \\ (\operatorname{GlcNAc-(Gal)}_a]_g^- & (\operatorname{Sia})_l^- & (R)_y \end{bmatrix}_u \\ (\operatorname{GlcNAc-(Gal)}_a]_h^- & (\operatorname{Sia})_m^- & (R)_y \end{bmatrix}_u \\ q_p$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0;

R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 42H

#### 187/345

```
Insect cell expressed DNase I.
a-d, f, h, j-n, s, u, v-y'=0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1,2,4,5, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1; j-n = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

#### FIG. 421

```
Yeast expressed DNase I.

a-n = 0;

q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- Endoglycanase
   Galactosyltransferase, UDP-Gal
- ↓ 3. CMP-SA-PEG, ST3Gal3

```
a-n, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

## FIG. 42J

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```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.
```

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated alpha-1-Proteinase inhibitor.
- 3, CMP-SA, ST3Gal3, ST3Gal1

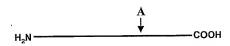
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker- alpha-1-Proteinase inhibitor.
```

## FIG. 42K



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \textbf{A} \leftarrow \text{-GlcNAc--} \\ \text{GlcNAc--} \\ \text{(R')}_{n} \end{array} \\ \begin{array}{c} \text{(GlcNAc--} \\ \text{(Gal)}_{b} \\ \text{[GlcNAc--} \\ \text{(Gal)}_{b} \\ \text{[GlcNAc--} \\ \text{(Gal)}_{b} \\ \text{[GlcNAc--} \\ \text{(Gal)}_{d} \\ \text{[GlcNAc--} \\ \text{(Gal)}_{d} \\ \text{[GlcNAc--} \\ \text{(Gal)}_{d} \\ \text{[GlcNAc--} \\ \text{(Gal)}_{d} \\ \text{(R')}_{p} \end{array} \\ \\ \text{(R')}_{n} \end{array}$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 43A

#### 190/345

```
CHO, BHK, 293 cells, Vero expressed Insulin.

a-m, r-u (independently selected) = 0 or 1;

n = 0; v-y = 0; z = 1.

1. Sialidase
2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1;

v-y (independently selected) = 1,
```

when j-m (independently selected) is 1;

n = 0; R = PEG; z = 1.

### FIG. 43B

```
Insect cell expressed Insulin.

a-h, j-n, s-y = 0;
i, r (independently selected) = 0 or 1; z = 1.

1. GNT's 1&2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
when e, g (independently selected) is 1;
```

## FIG. 43C

z = 1; R = PEG.

## 191/345

Yeast expressed Insulin.

a-n = 0; r-y (independently selected) = 0 to 1;

z=1;

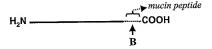
R (branched or linear) = Man, oligomannose or polysaccharide.

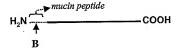
1. Endo-H

2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z=0; n = 1; R' = -Gal-PEG.

FIG. 43D





$$\mathbf{B} \quad \blacktriangleleft \begin{pmatrix} (\operatorname{Sia})_{b} \\ {}^{1} \\ \operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (\mathbb{R})_{d} \end{pmatrix}_{c}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer

## FIG. 43E

CHO, BHK, 293 cells, Vero expressed insulinmucin fusion protein.

a-c, e (independently selected) = 0 or 1; d = 0

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

## FIG. 43F

Insect cell expressed Insulin-mucin fusion protein. a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

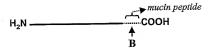
a, d, e (independently selected) = 0 or 1; b. c = 0; R = PEG.

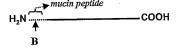
FIG. 43G

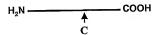
E. coli expressed Insulin-mucin fusion protein. a-e=0.

- GalNAc Transferase, UDP-GalNAc
   CMP-SA-PEG, sialyltransferase
- c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 43H







$$\mathbf{B} \quad \blacktriangleleft \stackrel{\mathrm{(Sia)}_{b}}{\left( \stackrel{\cdot}{\operatorname{GalNAc-(Gal)}_{a^{-}}(\operatorname{Sia})_{c^{-}}(\mathbb{R})_{d} \right)_{c}}}$$

a-c, e (independently selected) = 0 or 1; d=0; R=modifying group, mannose, oligo-mannose.

FIG. 431

E. coli expressed Insulin-mucin fusion protein. a-e,  $\mathbf{n} = \mathbf{0}$ .

 GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

## FIG. 43J

E. coli expressed Insulin-mucin fusion protein. a-e. n=0.

- GalNAc Transferase, UDP-GalNAc-linker-SA-CMP
   ST3Gal3, asialo-transferrin
- 3, CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

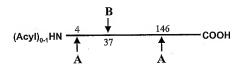
## FIG. 43K

## 197/345

E. coli expressed Insulin (N)—no mucin peptide. a-e, n = 0.

- NHS-CO-linker-SA-CMP
   ST3Gal3, asialo-transferrin
   CMP-SA, ST3Gal3
- a-e = 0; n = 1; R' = linker-transferrin.

FIG. 43L



$$\begin{array}{c} \mathbf{A} & \longleftarrow \begin{pmatrix} (\operatorname{Fuc})_i & \operatorname{Man} \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_a]_e^- & (\operatorname{Sia})_j - (R)_v \end{pmatrix}_r \\ -\operatorname{GlcNAc-GlcNAc-Man} & \operatorname{Man} \begin{pmatrix} [\operatorname{GlcNAc-(Gal)}_b]_r^- & (\operatorname{Sia})_k - (R)_w \end{pmatrix}_s \\ \operatorname{Man} & \left[ [\operatorname{GlcNAc-(Gal)}_d]_g^- & (\operatorname{Sia})_l - (R)_x \end{pmatrix}_t \\ \left[ [\operatorname{GlcNAc-(Gal)}_d]_h^- & (\operatorname{Sia})_m^- & (R)_y \end{pmatrix}_u \\ -\operatorname{GalNAc-(Gal)}_a - (\operatorname{Sia})_p^- & (R)_z \end{pmatrix}_{aa} \end{array}$$

a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 44A

# 199/345

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

 Sialidase
 CMP-SA-linker-lipid-A, ST3Gal3

```
a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = linker-lipid-A.
```

### FIG. 44B

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

- sialidase
- 2. CMP-SA-linker-tetanus toxin, ST3Gal1
- 3, CMP-SA, ST3Gal3

a-d, i-m, p-u, z, as (independently selected) = 0 or 1; o, v-y = 0; n, e-h = 1; R = tetanus toxin.

### 200/345

```
NSO expressed M-antigen.
a-d, i-n, o-u, aa (independently selected) = 0 or 1;
e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.
```

```
1. α-galactosidase
2. CMP-SA, ST3Gal3
2. CMP-SA-KLH, ST3Gal1
```

```
a-d, i-n, p-u, z, aa (independently selected) = 0 or 1;
e-h = 1; o, v-y = 0;
z = 1, when p = 1;
R = KLH.
```

# FIG. 44D

```
Yeast expressed M-antigen.
a-p, z = 0; q-y, aa (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

1. α1,2-mannosidase 2. GNT 1,

UDP-GlcNAc-linker-diphtheria toxin.

```
e, q, l, m, r, t, u, v, aa (independently selected) =0 or 1; a-d, f-h, j, k, n-p, s, w-z = 0; Sia = Man; R = linker-diphtheria toxin.
```

# **FIG. 44E**

#### 201/345

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

1. CMP-SA-levulinate, ST3Gal3, 2. H<sub>4</sub>N<sub>2</sub>-linker-DNA

a-d, i-m, o-y, as (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = linker-DNA.

### FIG. 44F

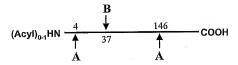
CHO, BHK, 293 cells, Vero expressed M-antigen a-d, i-n, o-u, aa (independently selected) = 0 or 1; e-h=1; v-z=0.

1. CMP-SA, poly-α2,8-ST

a-d, i, n-u, aa (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-100; y-z (independently selected) = 0.

# FIG. 44G

#### 202/345



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} \\ -\operatorname{GlcNAc}_{i} \\ -\operatorname{GlcNAc}_{i}$$

$$\mathbf{B} \quad \begin{array}{c} \text{(Sia)}_{\text{o}} \\ \text{-GalNAc-(Gal)}_{\text{n}}\text{-(Sia)}_{\text{p}}\text{- (R)}_{\text{z}} \\ \end{array}$$

a-d, i, n, q-u, aa, bb, (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-p (independently selected) = 0 to 100. Cc, v-y = 0; R = modifying group, mannose, oligo-mannose. R'= H, glycosyl residue, modifying group, glycoconiugate.

### FIG. 44H

# 203/345

```
Insect cell expressed M-antigen. a-d, f, h, j-m, o, p, s, u, v-z, cc = 0; bb = 1; e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

1. GNT-2, UDP-GlcNAc-linker-Neisseria protein

```
a, c, e, g, i, n, q, r, t, v, x, aa (independently selected) = 0 or 1;
b, d, f, h, j-p, s, u, w, y, z, cc = 0;
bb = 1; R = -linker-Neisseria protein.
```

### FIG. 441

```
Yeast expressed M-antigen.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

- Endoglycanase
- 2. Galactosyltransferase,
  UDP-Gal-linker-Neisseria protein

```
a-p, r-z, bb = 0;
q, aa, cc (independently selected) = 0 or 1;
R' = -Gal-linker-Neisseria protein.
```

# FIG. 44J

### 204/345

```
Yeast expressed M-antigen.
```

a-p, z, cc = 0;

q-y, aa (independently selected) = 0 to 1; bb = 1;

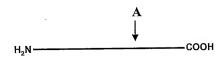
R (branched or linear) = Man, oligomannose;

GalNAc = Man.

- 1, mannosidases
- 2, GNT 1 & 2, UDP-GlcNAc
- 3. UDP-Gal, Galactosyltransferase,
- 4. CMP-SA, sialyltransferase

a, c, e, g, j, 1, q, r, t, aa (independently selected) = 0 or 1; b, d, f, h, k, m-p, s, u-z, cc = 0; bb = 1.

# FIG. 44K



$$(Fuc)_{i} \\ A \leftarrow -GlcNAc - GlcNAc - (Gal)_{a}l_{e} - (Sia)_{i} - (R)_{v} \\ - GlcNAc - (Gal)_{b}l_{f} - (Sia)_{k} - (R)_{w} \\ - (R')_{n} \\ (R')_{n} \\ (GlcNAc - (Gal)_{b}l_{f} - (Sia)_{k} - (R)_{w} \\ - (GlcNAc - (Gal)_{b}l_{f} - (Sia)_{h} - (R)_{v} \\ - (GlcNAc - (Gal)_{d}l_{h} - (Sia)_{m} - (R)_{y} \\ - (GlcNAc - (Gal)_{d}l_{h} - (Sia)_{m} - (R)_{y} \\ - (GlcNAc - (Gal)_{d}l_{h} -$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

### 206/345

CHO, BHK, 293 cells, Vero expressed Growth Hormone. a-m, r-u (independently selected) = 0 or 1; n = 0; v-y = 0; z = 1.

- Sialidase
   CMP-SA-PEG, ST3Gal3
- a-m, r-u (independently selected) = 0 or 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; n = 0; R = PEG; z = 1.

# FIG. 45B

```
Insect cell expressed growth hormone. a-h, j-n, s-y = 0; i, r (independently selected) = 0 or 1; z = 1.
```

1. GNT's 1&2, UDP-GlcNAc-PEG

```
a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected)= 0 or 1;
v, x (independently selected) = 1,
when e, g (independently selected) is 1;
z = 1; R = PEG.
```

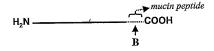
### FIG. 45C

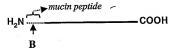
Yeast expressed growth hormone. a-n = 0; r-y (independently selected) = 0 to 1; z = 1; R (branched or linear) = Man, oligomannose or polysaccharide.

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z= 0; n = 1; R' = -Gal-PEG.

FIG. 45D





$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_b \\ -\mathrm{GaINAc-}(\mathrm{GaI})_a - (\mathrm{Sia})_c - (\mathrm{R})_d \end{bmatrix}_c$$

a-c, e (independently selected) = 0 or 1; d = 0; R = modifying group, mannose, oligomannose.

# FIG. 45E

### 209/345

CHO, BHK, 293 cells, Vero expressed growth hormone-mucin fusion protein. a-c, e (independently selected) = 0 or 1; d = 0

Sialidase
 CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

# FIG. 45F

Insect cell expressed Growth Hormone-mucin fusion protein.

a, e (independently selected) = 0 or 1; b, c, d = 0.

Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

### FIG. 45G

E. coli expressed growth hormone-mucin fusion protein. a-e=0.

1. GalNAc Transferase, UDP-GalNAc 2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

# FIG. 45H

E. coli expressed growth hormone-mucin fusion protein.

a-e, n = 0.

GalNAc Transferase,
 UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

FIG. 451

E. coli expressed growth hormone-mucin fusion protein.

a-e, n=0.

- GalNAc Transferase, UDP-GalNAc-linker-SA-CMP
   ST3Gal3, asialo-transferrin
- ▼ 3. CMP-SA, ST3Ga13

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

### FIG. 45J

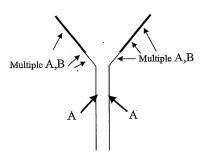
E. coli expressed growth hormone (N)—no mucin peptide.

a-e, n = 0.

- 1. NHS-CO-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

a-e=0; n=1; R'=linker-transferrin.

#### 212/345



$$A \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & \operatorname{Man} \left[ [\operatorname{GlcNAc-(Gal)}_{a}]_{o}^{-} (\operatorname{Sia})_{j}^{-} (\operatorname{R})_{v} \right]_{i} \\ -\operatorname{GlcNAc-Man} & \operatorname{Man} \left[ [\operatorname{GlcNAc-(Gal)}_{o}]_{g}^{-} (\operatorname{Sia})_{k}^{-} (\operatorname{R})_{w} \right]_{i} \\ -\operatorname{R'}_{i} & \operatorname{Man} \left[ [\operatorname{GlcNAc-(Gal)}_{o}]_{g}^{-} (\operatorname{Sia})_{i}^{-} (\operatorname{R})_{x} \right]_{t} \\ -\operatorname{R'}_{i} & \operatorname{GlcNAc}_{ww} & \left[ [\operatorname{GlcNAc-(Gal)}_{o}]_{g}^{-} (\operatorname{Sia})_{m}^{-} (\operatorname{R})_{y} \right]_{u} \\ -\operatorname{R'}_{i} & \operatorname{GlcNAc-(Gal)}_{m}^{-} (\operatorname{Sia})_{p}^{-} (\operatorname{R})_{zz} \right]_{q}$$

a-d, i-m, q-u, w, z, nn, ww, zz (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4.

n, v-y = 0;

R = modifying group, mannose, oligo-mannose;

R'=H, glycosyl residue, modifying group, glycoconjugate.

FIG. 46A

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

```
    CMP-SA, ST3Gal1
    galactosyltransferase, UPD-Gal
    CMP-SA-PEG, ST3Gal3
```

a-m, o-u, v-y, as (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

### FIG. 46B

CHO, BHK, 293 cells, Vero expressed TNF Receptor IgG Fusion. a-m, o-u, aa (independently selected) = 0 or 1; n=1; v-z=0.

1. sialidase
 ▼ 2. CMP-SA-PEG, ST3Gal1

a-i, p-u, z, aa (independently selected) = 0 or 1; n = 1; o, j-m, v-y = 0; R = PEG.

# FIG. 46C

#### 214/345

CHO, BHK, 293 cells, Vero expressed TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1; n = 1; v-z = 0.

1. galactosyltransferase, UPD-Gal-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

#### FIG. 46D

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion. a-m, o-u, aa (independently selected) = 0 or 1; n = 1;  $v \cdot z = 0$ .

- CMP-SA, ST3Gal1
- 2. galactosyltransferase, UPD-Gal-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

### FIG. 46E

```
CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion.

a-m, o-u, aa (independently selected) = 0 or 1;

n = 1; v-z = 0.
```

```
1. CMP-SA-levulinate, ST3Gal1
2. H<sub>4</sub>N<sub>2</sub>-PEG
```

```
a-m, o-u, v-y, as (independently selected) = 0 or 1;

n = 1; z = 0; R = PEG.
```

#### FIG. 46F

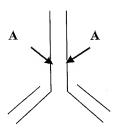
```
CHO, BHK, 293 cells, Vero expressed TNF Receptor IgG Fusion. a-m, o-u, aa (independently selected) = 0 or 1; n=1; v-z=0.
```

```
1. CMP-SA-PEG, \alpha2,8-ST
```

```
a-i, o, q-u, v-z, aa (independently selected) = 0 or 1; n = 1; j-m, p (independently selected) = 0 to 2; v-z (independently selected) = 1, when j-m, p (independently selected) is 2; R = PEG.
```

### FIG. 46G

### 216/345



$$\mathbf{A} \leftarrow \begin{bmatrix} [\operatorname{GlcNAc-(Gal)_a}]_{e^-} (\operatorname{Sia})_{j^-} (R)_{v} \end{bmatrix}_{r} \\ [\operatorname{GlcNAc-Man}]_{e^-} (\operatorname{GlcNAc-Man}]_{e^-} (\operatorname{Sia})_{k^-} (R)_{v} \end{bmatrix}_{r} \\ [\operatorname{GlcNAc-(Gal)_a}]_{e^-} (\operatorname{Sia})_{k^-} (R)_{v} \end{bmatrix}_{r} \\ [\operatorname{GlcNAc-(Gal)_a}]_{e^-} (\operatorname{Sia})_{e^-} (R)_{v} \end{bmatrix}_{v} \\ [\operatorname{GlcNAc-(Gal)_a}]_{h^-} (\operatorname{Sia})_{m^-} (R)_{v} \end{bmatrix}_{v} \\ [\operatorname{GlcNAc-(Gal)_a}]_{h^-} (\operatorname{Sia})_{m^-} (R)_{v} \end{bmatrix}_{v}$$

a-d, i, l, q-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-k (independently selected) = 0 or 1.

M = 0 to 20.

n, v-y=0; z=0 or 1;

R = polymer, toxin, radioisotope-complex, drug, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group, glycoconjugate.

**FIG. 47A** 

```
CHO, BHK, 293 cells, Vero expressed Herceptin. a, c, i (independently selected) = 0 or 1; e, g, r, t=1; b, d, f, h, j-m, n, s, u-y = 0; q, z=1.
```

galactosyltransferase, UPD-Gal
 CMP-SA-toxin, ST3Gal3

```
a, c, i, j, 1 (independently selected) = 0 or 1;
e, g, r, t=1; R = toxin;
f, h, k, m, n, s, u-y = 0; q, z = 1;
v-y (independently selected) = 51,
when j, 1 (independently selected) is 1.
```

### FIG. 47B

```
CHO, BHK, 293 cells, Vero or fungal expressed Herceptin. 
a, c, i (independently selected) = 0 or 1; 
e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0; 
q, z = 1.
```

 galactosyltransferase, UPD-Gal-Toxin

```
a, c, i (independently selected) = 0 or 1;
e, g, r, t=1; f, h, j-m, n, s, u-y=0;
q, z=1; v-y (independently selected) = 1,
when a, c (independently selected) is 1;
R = toxin.
```

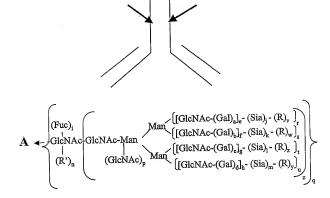
### FIG. 47C

Fungi expressed Herceptin. e, g, i, r, t (independently selected) = 0 or 1; a-d, f, h, j-m, n, s, u-y = 0; q, z = 1.

```
    Endo-H
    Galactosyltransferase, UDP-Gal
    GMP-SA-radioisotope complex, ST3Gal3
```

```
a-m, r-z= 0; q, n = 1;
R' = -Gal-Sia-radioisotope complex.
```

FIG. 47D



a-d, i, p-u, (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

i-m (independently selected) = 0 or 1.

n, v-y=0; z=0 or 1;

R = polymer, toxin, radioisotope-complex, drug, mannose, oligo-mannose.

R'=H, glycosyl residue, modifying group, glycoconjugate.

FIG. 48A

#### 220/345

```
CHO, BHK, 293 cells, Vero expressed Synagis.
a, c, i (independently selected) = 0 or 1;
e, g, r, t = 1;
b, d, f, h, j-m, n, s, u-y = 0; q, z = 1.
```

galactosyltransferase, UPD-Gal
 CMP-SA-PEG, ST3Gal3

```
a, c, i, j, w, (independently selected) = 0 or 1;
e, g, r, t = 1; f, h, k, m, n, s, u-y=0;
q, z = 1; v-y (independently selected) = 1,
when j, 1 (independently selected) is 1;
R = PEG.
```

### FIG. 48B

```
CHO, BHK, 293 cells, Vero or fungal expressed Synagis. a, c, i (independently selected) = 0 or 1; e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0; q, z = 1.
```

galactosyltransferase,
 UPD-Gal-PEG

```
a, c, i, w (independently selected) = 0 or 1;
e, g, r, t= 1; f, h, j-m, n, s, u-y= 0;
q, z= 1; v-y (independently selected) = 1,
when a, c (independently selected) is 1;
R = PEG.
```

Fungi expressed Synagis.

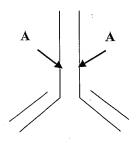
e, g, i, r,  $\hat{t}$  (independently selected) = 0 or 1; a-d, f, h, j-m, n, s, u-y = 0; q, z = 1.

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- → 3.. CMP-SA-PEG, ST3Gal3

a-m, r-z=0; q, n=1; R'=-Gal-Sia-PEG.

FIG. 48D

# 222/345



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_i \\ \operatorname{GlcNAc-Man} \\ | \\ (R')_n \end{bmatrix} \begin{bmatrix} (\operatorname{GlcNAc-(Gal)}_b]_e^- & (\operatorname{Sia})_j - (R)_v \end{bmatrix}_r \\ \left[ (\operatorname{GlcNAc-(Gal)}_b]_f^- & (\operatorname{Sia})_k - (R)_w \end{bmatrix}_g \\ \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_l - (R)_x \end{bmatrix}_t \\ \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_l - (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \\ \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u \right]_{u} \\ = \left[ (\operatorname{GlcNAc-(Gal)}_b]_g^- & (\operatorname{Sia})_m - (R)_y \end{bmatrix}_u$$

a-d, i, q-u, w (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 20.

n, v-y=0; z=0 or 1;

R = polymer, toxin, radioisotope-complex, drug, mannose, oligo-mannose.

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 49A

CHO, BHK, 293 cells, Vero expressed Remicade. a, c, i (independently selected) = 0 or 1; e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0; q, z = 1.

galactosyltransferase, UPD-Gal
 CMP-SA-PEG, ST3Gal3

```
a, c, i, j, 1 (independently selected) = 0 or 1;
e, g, r, t = 1; f, h, k, m, n, s, u-y = 0;
q, z = 1; v-y (independently selected) = 1,
when j, 1 (independently selected) is 1;
R = PEG.
```

#### FIG. 49B

CHO, BHK, 293 cells, Vero or fungal expressed Remicade. a, c, i (independently selected) = 0 or 1; e, g, r, t = 1; b, d, f, h, j-m, n, s, u-y = 0; q, z = 1.

galactosyltransferase,
 UPD-Gal-PEG

```
a, c, i (independently selected) = 0 or 1;
e, g, r, t=1; f, h, j-m, n, s, u-y=0;
q, z=1; v-y (independently selected) = 1,
when a, c (independently selected) is 1;
R = PEG.
```

#### FIG. 49C

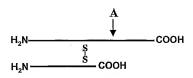
Fungi expressed Remicade.

e, g, i, r, t (independently selected) = 0 or 1; a-d, f, h, j-m, n, s, u-y = 0; q, z = 1.

- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal
- → 3.. CMP-SA-radioisotope complex, ST3Gal3

a-m, r-z= 0; q, n = 1; R' = -Gal-Sia-radioisotope complex.

FIG. 49D



$$\mathbf{A} \leftarrow \begin{bmatrix} [\operatorname{GlcNAc-(Gal)_s]_e^-(Sia)_j^-(R)_v]_r \\ [\operatorname{GlcNAc-(Gal)_s]_e^-(Sia)_k^-(R)_w]_s \\ [\operatorname{R''})_n \end{bmatrix} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)_s]_e^-(Sia)_j^-(R)_v]_r \\ [\operatorname{GlcNAc-(Gal)_s]_g^-(Sia)_l^-(R)_w]_s \\ [\operatorname{GlcNAc-(Gal)_s]_g^-(Sia)_l^-(R)_w]_u \end{bmatrix} \begin{bmatrix} [\operatorname{GlcNAc-(Gal)_s]_e^-(Sia)_j^-(R)_w]_s \\ [\operatorname{GlcNAc-(Gal)_s]_g^-(Sia)_l^-(R)_w]_u \end{bmatrix} \end{bmatrix}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 50A

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```
CHO, BHK, 293 cells, Vero expressed Reopro. a-m, r-u (independently selected) = 0 or 1; n=0;\ v-y=0;\ z=1.
```

```
    Sialidase
    CMP-SA-PEG, ST3Gal3
```

```
a-m, r-u (independently selected) = 0 or 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
n=0; R=PEG; z=1.
```

#### FIG. 50B

```
Insect cell expressed Reopro. a-h, j-n, s-y = 0; i, r (independently selected) = 0 or 1; z = 1.
```

1. GNT's 1&2, UDP-GlcNAc-PEG

```
a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
when e, g (independently selected) is 1;
z = 1; R = PEG.
```

# FIG. 50C

#### 227/345

```
Yeast expressed Reopro.
```

a-n=0; r-y (independently selected) = 0 to 1;

z=1;

R (branched or linear) = Man, oligomannose or polysaccharide.

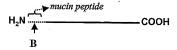
1. Endo-H

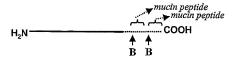
2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z=0; n = 1; R' = -Gal-PEG.

### FIG. 50D







a-c, e (independently selected) = 0 or 1; d = 0; R = polymer

# FIG. 50E

CHO, BHK, 293 cells, Vero expressed Reopro-mucin fusion protein. a-c, e (independently selected) = 0 or 1; d = 0

> 1. Sialidase 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

# FIG. 50F

Insect cell expressed Reopro-mucin fusion protein. a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

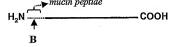
FIG. 50G

E. coli expressed Reopro-mucin fusion protein. a-e=0.

- GalNAc Transferase, UDP-GalNAc
   CMP-SA-PEG, sialyltransferase
- c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 50H





$$\mathbf{B}$$
  $\leftarrow$   $\begin{pmatrix} (\mathrm{Sia})_b \\ \mathrm{GaINAc-(Gal)_a-(Sia)_c-(R)_d} \end{pmatrix}_c$ 

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer, linker.

FIG. 501

E. coli expressed Reopro-mucin fusion protein. a-e, n=0.

 GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

### FIG. 50J

E. coli expressed Reopro-mucin fusion protein. a-e, n=0.

- GalNAc Transferase,
   UDP-GalNAc-linker-SA-CMP
   ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Ga13

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

### FIG. 50K

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E. coli expressed Reopro(N)-no mucin peptide. a-e, n=0.

- 1. NHS-CO-linker-SA-CMP
- ST3Gal3, asialo-transferrin
   CMP-SA, ST3Gal3

a-e=0; n=1; R'=linker-transferrin.

FIG. 50L